4.3 Data relevant to comparisons across agents and endpoints

4.3.1. General description of the database

A vast amount of high throughput screening (HTS) data has been generated as part of the government interagency programs in the USA that are known as Tox21 and ToxCast research programs. The US EPA has systematically analysed over 1 million concentration response sample-assay pairs from ToxCast and Tox21. The resulting concentration response models and activity calls have been publicly released via the Chemical safety for Sustainability (CSS) ToxCast Dashboard (www.actor.epa.gov/dashboard). Summary matrix files, the toxcast data analysis pipeline (tcpl) R package and connected database (invitrodb_v1) have also been publicly released (www.epa.gov/toxcast/data). Summary matrix files are consistently outputted with rows of chemicals, columns of assay endpoints and intersection of various models parameters (e.g., logAC50, top of curve), activity call, testing status or z-scores (i.e., potency distance from cytotoxicity burst). The tcpl R package and associated database enables access to all of the underlying concentration response data, the analysis decision logic and methods, concentration response model outputs, activity calls and activity caution flags.

Overall, Tox21 and ToxCast research programs have tested over 8000 and 1800 chemicals, respectively. Specifically, ToxCast has tested about 1000 chemicals across the full assay battery comprising over 800 *in vitro* tests. The remaining 800 chemicals were tested as part of an endocrine profiling effort that resulted in a subset of these assays. Within the ToxCast chemical library, over 30 organophosphate pesticides or their oxon metabolites were tested across the entire assay battery, including Diazinon, Malathion, Parathion, and Tetrachlorvinphos as well as three oxon metabolites, Diazoxon, Malaoxon and Paraoxon. Glyphosate was not included in either of the chemical libraries due to physico-chemical property constraints.

Data on 821 assay endpoints derived from 558 assay components (i.e., readouts) and 342 assays (i.e., experiments) are available in the US EPA dashboards. The 342 assays were sourced from 7 vendors or collaborators spanning diverse technological and biological space, including over 300 gene targets. About half of the final assay endpoints were analysed from cell-free assay formats with the remainder from cell-based assays. It is of note that while the cell-based assays have a variable metabolic capacity, it is generally limited.

4.3.2. Alighning *in vitro* assays to 10 "key characteristics" of known human carcinogens

In order to explore the bioactivity profiles of the compounds under evaluation in Monograph 112 with respect to their potential impact on the mechanisms of carcinogenesis, the Working Group members performed mapping of the 821 available assay endpoints in Tox21/ToxCast to 10 Key Characteristics of known human carcinogens (REF to IARC instructions for Section 4 table). Independent assignments were made for each assay type to the one or more "key characteristics" based on the biological target being probed by each assay and the interpretation of the assay read-out. The consensus assignments comprise 274 assays that mapped to 7 "key characteristics" as shown below.

1. Is Electrophilic or Can Be Metabolically Activated – 81 assay endpoints
2. Is Genotoxic – 14 assay endpoints
3. Alters DNA repair or causes genomic instability – 0 assay endpoints
4. Induces Epigenetic Alterations – 18 assay endpoints
5. Induces Oxidative Stress – 34 assay endpoints
6. Induces chronic inflammation – 48 assay endpoints
7. Is Immunosuppressive – 0 assay endpoints
8. Modulates receptor-mediated effects – 143 assay endpoints

9. Causes Immortalization – 0 assay endpoints

10. Alters cell proliferation/death or nutrient supply – 157 assay endpoints

The match of an assay to the "key characteristic" were to provide insight into the bioactivity profile of a chemical highlighting the chemical's potential to interact or disrupt targets biologically associated with cancer. In addition, based on the in vitro assays that represent each "key characteristic", a comprehensive and unbiased evaluation of the relative potency of each compound under evaluation may be performed. For each assay, an activity concentration (*i.e.*, activity concentration exceeding +/-3 mean absolute error variability over the baseline in each assay) for active or 0 for inactive (assays where at no concentration tested the signal exceeded the threshold of +/-3 mean absolute error variability over the baseline) were derived. To integrate the data across individual assays into the cumulative signal for each "key characteristic", the ToxPi software (Reif et al. 2013) was used. ToxPi is an example of a prioritization support tool for integration of evidence across endpoints and to visualizing the relative prioritized ranks of the compounds under consideration. ToxPi was proposed by (Reif et al. 2010) as a dimensionless index score that enables integration of multiple sources of evidence on exposure and/or safety, transformed into transparent visual rankings to facilitate decision making. Different data are translated into ToxPi scores to derive slice-wise scores for all compounds as detailed below and in the publications describing the approach (Reif et al., 2010) and the associated software package (Reif et al., 2013). The individual slice values were further normalized from 0 to 1 based on the range of responses for each slice across all 1000 chemicals. The Toxicological Prioritization Index (ToxPi) visualization of the CKC assay endpoint groupings highlighted each chemical's relative potential to interact with the set of assay endpoints per grouping (Reif et al 2010).

4.3.3. Malathion-specific effects across 7 "key characteristics" based on in vitro screening data.

Relative effects of malathion and malaoxon were evaluated compared to 180 IARC-evaluated chemicals that also were screened by Tox21/ToxCast program. Of the 180 chemicals, 8 were Group 1, 16 were Group 2A, 58 were Group 2B, 97 were Group 3, and 1 was Group 4.The results are presented as a ranked list of all compounds in the analysis (180 IARC-classified chemicals and monograph volume 112 compounds) arranged in the order of the relative effects (most active, i.e., effects observed at the lowest concentration, to least active, no effect observed in any concentration tested or effects at the highest concentrations tested) and the relative position of malathion and malaoxon are also shown. In addition, a ToxPi graph of the sub-categories that comprise assays in each characteristic are also shown in a form of a pie chart.

(d) "**Oxidative Stressor.**" There were 18 assays mapped to this characteristic in sub-categories of Metalloproteinase (5), Oxidative stress (7), and Oxidative stress marker (6). As it can be observed from the analysis, malathion exhibits intermediate potency based on the results of these in vitro tests, as compared to most potent agents benzo(b)fluoranthene and 4-chloro-1,2-diaminobenzene.